D. KRÜGER & A. GARGAS

The basidiomycete genus *Polyporus* – an emendation based on phylogeny and putative secondary structure of ribosomal RNA molecules

With 4 Figures and one Table

Summary
The fungal genus *Polyporus* is an assemblage of white-rotting lignicolous basidiomycetes. It has undergone considerable expansion and contraction over a period of two and three quarter centuries. Current generic circumscription of *Polyporus* has kept the genus non-monophyletic. Species of *Polyporus* infrageneric group *Polyporellus* are closely related to some species of *Lentinus*. We introduce data for ITS2 spacer rRNA second structure evolution by quasi-independent comparison with large subunit rRNA phylogeny, and suggest a fraction of primary nuclear rDNA ITS sequence data as novel taxonomic character. A major taxonomic shift is suggested, supported by molecular and morphological characters, and allowing inclusion of species with gilled hymenophores in *Polyporus*. Two new names are proposed: *Polyporus phyllostipes* D. KRÜGER, nom. nov. and *Polyporus gerdai* D. KRÜGER, nom. nov.

1 Introduction

One of the more conspicuous genera of pore fungi, *Polyporus* (Basidiomycotina) has been in use since 1729 (MICHELI 1729). The name represents a basidiomycete alliance with a history of periodic contraction and expansion. When ADANSON (1763) took up MICHELI's name *Polyporus*, he omitted reference to a figure (Pl. 71, Fig. 1) later selected as the iconotype for the *Polyporus* generic type species *P. tuberaster* by DONK (1960: 261). Circa 1812, PAULET (Icon. Champ.) used the name *Polyporus* in seven binomials, with *P. ulmi* PAULET the first name mentioned.

FRIES (1821: 341), in sanctioning *Polyporus* as "*Polyporus MICHELI p. 129*", applied the name to almost all polypores, leaving *Fistulina* BULL. and *Daedalea* PERS. separate. Published by NÚÑEZ & RYVARDEN (1995), the latest
monographic treatment contained only 32 species. They were arranged in six infrageneric groups that were not given ranks. Those groups were Dendropolyergus, Polyergus s. str. (= Squamosus group), Polyporellus, Melanoporus, Admirabilis, and Favolus. Several species have been added or resurrected more recently (Buchanan & Ryvarden 1998; Dai 1996, 1999; Hattori 2000; Popoff & Wright 1998; Thorin 2000).

The following lectotypifications have been proposed: i) Murrill (1903) first selected a lectotype for Polyergus (Micheli) Paulet: Paulet’s first species, P. ulmi Paulet [Icon. Champ.: Pl. 13 (1793) – Donk (1960: 261) gave the date as 1812–1835]. Murrill (1903) regarded P. ulmi as synonymous with P. squamosus Huds.: Fr. and P. caudicinus Scop.: Fr. Admittedly using the then fashionable “first-species-rule” which has been judged mechanical (ICBN St. Louis Code by Greuter et al. 2000: 12 Art. 9.2 and 16 Rec. 9A.2.), this lectotypification is not tenable. ii) Clements & Shear (1931: 347) selected P. brunalis (Pers.) Fr. as type of Polyergus (Mich.) Fr. They chose the type “from the best known or more important species ... in order to avoid change and ensuing confusion as far as possible” [Clements & Shear (1931: 15); an argument also used by Redhead & Ginn (1985) to defend a Clements & Shear typification of Lentinus (see below)]. Polyergus brunalis is the sixth species in Fries (1821: 348) of “Polyergus Micheli.” B. Microporus Trib. I. Mesopus. Clements & Shear (l. c.) referred to Polyergus as “Polyergus (Micheli) Fr. Epirc. 427 (1838),” where one finds Fries himself referring to “S. M. p. 341” (i.e. Fries 1821: 341) and giving P. brunalis as twelfth species (Epircis. Syst. Mycol.: 430). Thus, Clements & Shear certainly did not use mechanical means of selecting types, making their proposed lectotype the first acceptable type for Polyergus. iii) Donk (Meded. Nederl. Mycol. Ver. 22: 124–126, 1933) instead selected P. tuberaster as a species common to Micheli’s, Paulet’s, and Fries’ sense of Polyergus (see Donk 1960: 262). Donk (1960: 263) dismissed Clements & Shear’s lectotypification, as he considered P. brunalis as only “doubtfully represented” among species illustrated by Micheli. Dismissal of the valid 1931 lectotypification based on this argument is not supported by the ICBN (Greuter et al. 2000: 12 Art. 9.2 and 16 Rec. 9A.2.), as any species included by Fries in the sanctioning volumes may be selected as type. iv) Without expressed justification, Cunningham (1948: 1) proposed P. arcularius (Batsch) Fr. as type, but this is later than Clements & Shear’s proposal. The species names Polyergus arcularius, P. brunalis, and P. ciliatus have been misapplied in the past (Kreisel 1963), perhaps having led to P. arcularius being stated as type by Cunningham (1948).


Reference of P. tuberaster as type was based on three arguments: i) P. tuberaster was a once cultivated organism deserving name stability (Kreisel 1960), ii) rejection of automatic first-name-rule typification (see Núñez 1993; Núñez & Ryvarden 1995), and iii) stability of the use of the name Polyergus. Corner (1984: 11) challenged P. tuberaster as Polyergus lectotype, instead opting for P. squamosus Huds.: Fr., reporting that P. squamosus had the typical hyphal construction of Polyergus s. str., and that P. tuberaster

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was poorly known. These taxonomic opinions cannot disqualify *P. brumalis* as first selected lectotype. DONK (1960: 261) suggested that *P. tuberaster* and *P. squamosis* might be conspecific, which has been refuted by mating studies by NÜNEZ (1995) and molecular data (HIBBETT & DONOGHUE 1995; KRÜGER 2002: e.g. 25, 150; KO & JUNG 2002a).

The close relationship of *P. (Polyporellus)* *arcularius* (Polyporaceae CORDA 1839) and the gilled mushrooms of *Lentinus* subgen. *Lentinus* (Lentinaceae JÜLICH 1981) was confirmed by HIBBETT & VILGALYS (1993), HIBBETT & DONOGHUE (1995), and KO & JUNG (2002a), although such positional proximity within the weakly resolved / Polyporoid [the "F" indicates a monophyletic clade, as in BAUM et al. (1998), MONCALVO et al. (2002) and THOMAS et al. (2002)] was not seen in HIBBETT & DONOGHUE'S (2001) consensus tree. HIBBETT & VILGALYS (1995) presented evidence for *Lentinus* being derived from polypores, as postulated by PEGLER (1983: 11). KO & JUNG (2002a) suggested that *Polyporus sensu* NÜNEZ & RYVARDEN (1995) was not monophyletic based on mitochondrial rDNA sequences. So far, no taxonomic arrangements to reflect this inferred kinship of *Lentinus* and *Polyporus* have been proposed.

The aim of this study was i) to investigate *Lentinus* and *Polyporus* species relationship, as evidenced by nuclear large subunit DNA data; ii) to compare the putative secondary structure of spacer nuclear ribosomal RNA and define a consensus structure for taxonomic characters independent of large subunit primary sequence, and iii) to derive a practicable taxonomic and nomenclatural conclusion for *Lentinus* and *Polyporus*.

### 2 Materials and methods

#### Collections and microscopy

Collections (see Table I) were given field book numbers and annotated. Specimens were deposited in the University of Tennessee fungal herbarium (TENN). Identification was done using keys furnished by JÜLICH (1984), GILBERTSON & RYVARDEN (1986–1987), RYVARDEN & GILBERTSON (1993–1994), and NÜNEZ & RYVARDEN (1995).

#### DNA extraction

DNA was extracted from herbarium specimens or cultures following protocols described by KRÜGER et al. (2003, 2004), using a modified xanthogenate-based procedure (TILLETT & NEILAN 2000). With culture material, a short centrifugation step and the removal of some liquid was done before proceeding to grinding. Mechanical cell disruption was carried out in 50 μl TE extraction buffer or after the addition of the xanthogenate buffer.

#### PCR and sequencing

Amplification of the nuclear ribosomal large subunit (nLSU) gene was conducted with primers ITS 5 (WHITE et al. 1990) and LR 7 or LROR/LR 7 (www.biology.duke.edu/fungi/mycolab/primers.htm). PCR parameters for the primer pair LROR and LR 7 (20 μL reactions) were as follows: initial denaturation 94°C for 3 min, followed by 37 cycles of denaturation 94°C for 1 min, annealing 46°C for 1 min, extension 72°C for 3 min, with adjustments when amplifying with primers ITS 5 and LR 7. Subsequent LROR and Nu-LSU335-5′ (CTAAATTTGGGAGAGAC ; *T*~m~ = 53.88°C after calculation at www.genosys.com) cycle sequencing reactions (10 μl) contained 2 or 3 μl BigDye v. 2.0 reaction mix, 0.32 μl 10 μl primer, and approximately 25 ng template DNA. Excess primers and nucleotides were removed by an ethanol precipitation protocol: adding 10 μl 1 dd H2O, 50 μl 95% ethanol, and 2 μl 3 M sodium acetate (pH 5.2) to the cycle sequencing products; precipitation at room temperature for 20 min. Next, samples were spun for 20 min (16,000 g). Supernatant was removed, and the pellet was washed with 190 μl 70% ethanol and recentrifuged (5 min, 16,000 g). The supernatant alcohol was pipetted off, and the pellet was dried by incubation for 1 min at 90°C. Sequencing reactions were analyzed on Perkin-Elmer automated sequencers at the University of Tennessee sequencing facility.

#### Phylogenetic analyses

Sequences obtained with electropherograms were corrected in Chromas v. 1.45 (Technelysium Pty. Ltd., Australia), and assembled using a text editor. One additional sequence was imported from GenBank (X78776, MONCALVO et al. 1995). ClustalX v. 1.64b (THOMPSON et al. 1997) and BioEdit v. 5.0.9 (HALL 1999) were used in alignment. The data used here represent a subset of a dataset in KRÜGER (2002), containing 24 taxa and 724 aligned characters. All trees were *a-priori* rooted with Hallenberg 2518-1 *Neolentinus maculassimus*, and processed in TREEVIEW v. 1.6.1. (PAGE 1996).
Table 1
Fungal material used for phylogenetic and Mfold analyses

<table>
<thead>
<tr>
<th>Strain numbers and/or herbarium voucher numbers if known</th>
<th>Fungal species and authors</th>
<th>Country of origin</th>
<th>Names of collectors and identifiers</th>
<th>GenBank number and study</th>
<th>NÚNEZ &amp; RYVARDEN (1995) group</th>
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<td>FB10126 SBI 2 (TENN57346)</td>
<td><em>Ganoderma tsugae</em> MURRILL</td>
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Table 1 (continued)

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FB = TENN field book number = CultENN culture collection number.
SBI = single basidiospore isolate.
TENN = Univ. of Tennessee Fungal Herbarium, other herbarium acronyms from HOLMGREN et al. (1981)

Maximum parsimony

The program SEPAL (SALISBURY 2001) was used for a maximum-parsimony analysis with jackknife resampling procedure (EFRON & GONG 1983; 100 pseudoreplicates, 20% deletion, 50% majority rule). In addition, branch support was estimated by a calculation of decay indices (BREMER 1994). PAUP* v. 4.0b10 was also used to TBR-swap on the decay tree of SEPAL. All most-parsimonious trees (MPTs) found in this round, together with the SEPAL-generated tree, were combined into one 50% majority rule tree and displayed with indicated bipartition frequencies over 50% achieved in the jackknife analysis. All trees were also compared in their minimum-evolution (ME)-score, under the Kimura-2 parameter model.

Bayesian posterior probabilities

Bayesian likelihood analyses were performed in MrBayes (HUelsenbeck & Ronquist 2001), including parameter optimization on a general-time-reversible (GTR) likelihood model of sequence evolution with gamma-shaped distribution of rate heterogeneity. The analysis settings were (after doing three independent analyses to decide on settings): 100,000 generations with a random starting tree, burn-in threshold of 10,000 trees, sampling frequency of 500. Six search chains of Markov Chain Monte Carlo were run in parallel. PAUP* v. 4.0b10 (SWOFFORD 2001) was used to examine the sampled trees from Bayesian analysis and to calculate an 80% majority rule consensus tree. Bipartitions with over 60% support are indicated in Fig. 2.
Puzzle likelihood

Puzzle tree-reconstruction (STRIMMER & VON HASELER 1996; STRIMMER et al. 1997) was run assuming a gamma-distributed rate heterogeneity (eight categories; approximated shape parameter, nucleotide frequencies, and transition/transversion bias estimated from the data), HKY model (HASEGAWA et al. 1985), neighbour-joining tree as starting tree.

Likelihood-based tests

The Kishino-Hasegawa (KISHINO & HASEGAWA 1989) and Templeton (TEMPLETON 1983) tests implemented in PAUP* were utilized to compare all parsimony trees from SEPAL and PAUP*, as well as the Bayesian likelihood consensus tree and the Puzzle likelihood tree, in the significance of parsimony length differences. As the compared trees do not include a most-likely tree, these statistical tests do not fall under the circumstances described as improper by GOLDMAN et al. (2000).

Displaying trees

Trees were prepared for publication with TREEVIEW v. 1.6.1. (PAGE 1996) and data and trees were archived in TreeBASE (Study Number S1113).

ITS2 secondary structure generation

Secondary structure of rRNA spacers is important in ribosome assembly and thus must be under evolutionary constraints (LALBEV & NAZAR 2001). ITS2 rDNA boundaries were distinguished by comparison to published sequences for Saccharomyces cerevisiae; the 3.8S DNA region to RUBIN (1973), and the LSU rDNA region to BAYEV et al. (1981). Folding for ITS2 rRNA transcript was performed by MFold version 3.1 (ZUKER 2003) using free energy calculations (default conditions: linear RNA sequence, folding temperature of 37 °C, 20% suboptimal, upper bound of 50 foldings, no limit to the maximum distance between paired bases, maximum number of nucleotides in a bulge or loop = 30, maximum asymmetry of an interior/bulge loop = 30). From the initially predicted foldings, those most closely resembling the four conserved pairing regions (stems) hypothesized for plants and green algae (MAI & COLEMAN 1997), dinoflagellates (GOTTSCHILING & PLÖTNER 2004) and various unrelated fungi (GARGAS & KRÜGER unpublished) were carefully scrutinized. We then repeated the foldings with shorter stretches of primary sequence data to obtain only the first two of these major stem-loop structures, termed P1 and P2 hereafter. This was an iterative process stopped once full ITS2 rRNA and partial ITS2 rRNA foldings inferred identical structures for the P1 through P2 part of ITS2.

Comparing putative ITS2 structure with LSU phylogeny

The 5′ half of the 28S large subunit rRNA of the pre-rRNA transcript that is encoded by the data used for phylogenetic analyses is assumed not to interact on the secondary structural level with the ITS2 spacer pre-rRNA segment. Thus, we assume that the sequences analyzed here for phylogeny and for putative secondary structure are largely independent. This allows the postulating of hypothetical events in ITS structure evolution when assuming the correctness of the phylogenetic trajectory indicated by LSU data.

Nomenclature

For nomenclature questions, we consulted the current ICBN Code (GREUTER et al. 2000, www.hgbm.fu-berlin.de/iapt/nomenclature/code/SaintLouis/ 0005LStLouislike.htm). Synonymy was checked with various taxonomic literature cited throughout, as well as using online databases (www.indexfungorum.org; STALPERS 2004).

3 Results

Modeltest

The general-time-reversible (GTR + G + I, − ln L = 2,966.429) model was selected as best by Modeltest (POSADA & CRANDALL 1998), but in Puzzle we chose to use the computationally less demanding HKY model (− ln L = 3,109.048) for likelihood assessments among available models.

Maximum parsimony

The data included 74 variable but parsimony-uninformative characters and 94 parsimony-informative characters. Swapping on the SEPAL tree (174,560 TBR rearrangements) generated another 38 most-parsimonious trees (MPT) in PAUP*. Each had a length of 384 steps (207 to 624 steps possible), CI = 0.539, and RC = 0.310. The other parsimony trees scored with: 405 steps / CI = 0.511 / RC = 0.268 / ME-score = 0.52888 (SEPAL), and 421 steps / CI = 0.492 / RC = 0.268 / ME-score = 0.53952 (SEPAL jackknife: 20% deletion).
Bayesian posterior probabilities

180 trees were used to compute the 80% majority rule consensus tree of the Bayesian analysis. The consensus tree had the following parameters: 405 steps / CI = 0.511 / RC = 0.268 / ME-score = 0.53994. It was identical to the SEPAL tree in parsimony parameters.

Puzzle likelihood

10,626 quartets (19.4% unresolved) were analyzed, 1,888 site patterns found, and the shape parameter estimated as alpha = 0.12. The estimated transition/transversion ratio was 3.51 and the estimated pyrimidine transition/purine transition ratio was 0.62. After thirteen iterations, likelihood converged on -ln L = 3,009.90. The Puzzle tree scored (in PAUP*) with 416 steps / CI = 0.498 / RC = 0.248 / ME-score = 53,461.

Likelihood-based tests

The likelihood-based tests applied to nucleotide character change steps rejected all non-parsimonious trees.

Tree depiction

Shown herein are the following trees: the Puzzle tree (Fig. 1) with Puzzle bipartition supports, the Bayesian consensus (Fig. 2 left) with branching supports over 50 indicated, and one of the MPT (indicated as best in the likelihood-based tests) with decay indices and jackknife bipartition supports (Fig. 2 right).

Secondary structure

Each of the sequences used resulted in only one structure (Figs. 3, 4) deduced with standard Mfold settings. Note that the placement of the basal base pair of the major stem-loops, and the size of the joiner between them, depends on the extent of sequence data pasted into Mfold.

4 Conclusions

Observed and inferred phenotypic characters

The inflated generative hyphae as depicted by PEGLER (1983: e.g. p. 47 for L. tigrinus) for Lentinus s. str. (subgen. Lentinus, section Ti-grini), and also found prominently in many young specimens of Polyporellus by us (KRÜGER 2002) appear to be a unifying character for a Lentinus-Polyporellus alliance perhaps in addition to hyphal pegs as suggested by HIBBETT & VILGALYS (1993). PEGLER (1983: 5) mentioned the sclerotia/pseudosclerotia of Lentinus, which are also found in Polyporus (Squamosus and Dendropolyergus groups) and Laccocephalum MCAIP. & TEPPER (NÚNEZ 1995; NÚNEZ & RYVAR DEN 1995). It remains to be determined whether the formation of sclerotia arose independently in the Lentinus/ Polyporellus alliance and in other polypores.

Polyporellus spp. differ from other Polyporus spp. in spore sizes (Polyporus group Squamosus spores are longer, see NÚNEZ & RYVAR DEN 1995) and existence of widely inflated generative hyphae, especially tropical specimens of P. tricholoma, and specimens fruiting on sawdust (KRÜGER et al. 2003). Central lengths of skeletal-binding hyphae also can take the inflated form, with a varying width of lumen in the wider part. Abrupt changes from generative to skeletal hyphae or vice versa can be seen at a clamp connection. Sometimes one can find irregularly-shaped clamp connections that also are branching points. Other features typical for group Polyporellus may be monokaryotic fruiting (HOFFMANN 1978), and the sometimes fuzzy hirsute appearance of entire fruit bodies or caps (KREISEL 1963; BREMER 1986). Other basidiomata of P. brumalis and P. ciliatus develop reddish or ochraceous stains, occasionally impeding easy recognition in the field. Fuzzy and ciliate pilear surface and margin also can be found in species of Lentinus (e.g. compare PEGLER 1983: 30, 32, 38).

Mfold inference of ITS2 structures was influenced by ambiguity codes in the sequences of Polyporus arcularius, P. brumalis, and P. tricholoma. In particular, ambiguity lead to additional loops (e.g. ambiguity replaced by boxed N, see Fig. 3). We could not judge whether there were non-orthologous rDNA or non-functional divergent copies (KO & JUNG 2002b; KRÜGER et al. 2004; RAZAFIMANDIBISON et al. 2004), or whether PCR or sequencing artifacts led to the ambiguity. We drew binding indicators across the loops generated by the nucleotide ambiguity (Fig. 3). ITS hy-
pervariability due to microsatellites has recently found renewed interest in fungal molecular phylogenetics (MIADLIKOWSKA et al. 2003; DEN BAKKER et al. 2004), and there is urgent need to re-evaluate secondary structure and concerted evolution of rDNA repeats. As multi-gene phylogenies are accumulating, refining the models of sequence evolution of non-protein coding sequences, e.g. involving secondary structure information (e.g. SMITH et al. 2003), appears feasible. This is also necessary in light of lack of resolution in single-gene phylogenies (ROKAS et al. 2003) and finding break points of chimerics in environmental rDNA PCR clone libraries (HUENHOLTZ & HUBER 2003).

Taking the LSU phylogeny as trajectory for the evolution of inferred ITS2 rRNA foldings,
Bayesian Likelihood

8744-7 Pseudotavulus coccinnatus
11279 Mycobionia flavum
10197 Polyporus tuberaster
10531 Polyporus squamosus
10662-19 Polyporus versus
10459-10 Polyporus triperti
10221-8 Polyporus guianensis

68** for bipartition

87

94

100

87

94

88

92

83

91

92

95

94

89

99

78** for bipartition containing three taxa

Maximum Parsimony

68, 1

95, 2

86, 1

62, 0

92, 5

0, 1

0, 1

76, 0

98, 6

75, 1

0, 0

75, 1

68, 0

56, 0

one can formulate patterns of character evolution (Fig. 4, based on Puzzle tree Fig. 1) within /Tigrinus-Polyporellus. Starting from an unspecified common ancestor of both /Trametes and /Tigrinus-Polyporellus, a two-sided interior loop subterminally located in the first major stem-loop of ITS2 (labeled P1) at the base of boxed element 1 disappeared somewhere basal to the included Polyporellus and Lentinus taxa (hypothetical event a). Event b, T/C and G/C point mutations for element 2 of P. tricholoma, allows for a structural synapomorphy. The enlargement of the terminal loop of P2 (element 3) appears to be related to a hypothetical insertion of an A in the coding DNA in the ancestor of P. ciliatus (event c). Event d appears to include several point mutations affecting the primary sequence within element 3 which cannot accurately be placed on a branch. The one structural element that seems consistent with the suggested phylogeny is the loss of the interior loop associated with event a, allow-
Lentinus tigrinus

Polyporus tricholoma

Polyporus arcularius

Polyporus brumalis

Polyporus ciliatus

Trametes hirsuta

Fig. 3
Six secondary structures of the first two major pinloops (left hand: P1, right hand: P2) of ITS2 rRNA transcripts as inferred by Mfold
Fig. 4. Derived putative diagnostic structures of ITS 2 RNA (P1, P2) for six taxa of *Tigrinus-Polyporus* and one outgroup *Trametes* with hypothetical events based on a takeout (partial phylogeny) of Fig. 1. The three discussed example structure elements are labeled 1, 2, and 3 (boxed). Hypothesized events labeled on internodes are explained here at the DNA level. Event a = potential switching of T versus C and G versus C in DNA coding for element 2 in *P. iricholomae*. Event c = insertion of an A enlarging the terminal loop in element 3 of *P. ciliatus*. Event d = point mutations in DNA encoding terminal loop in element 3, no defined internodal position of event. Nucleotides affected by these events highlighted in shaded boxes.
potheses contains the majority of included/core-Polyporus taxa: Puzzle: 80%, Bayesian: 100%, 92% parsimony jackknife support, decay index of 5. Publications based on results in KRÜGER (2002) are underway to address further problems of Polyporus evolution and systematics.

Descriptions

Polyporus ADAMSON (Fam. Pl. 2: 10. 1763); FRIES, Syst. Mycol. 1: 341 (1821) emend. D.KRÜGER

Genus accepted as circumscribed by NÜNEZ & RYVARD (1995), but including stipitate, wood-decaying fungi with dimitic hyphal construction [generative and skeletobinding hyphae, both can be inflated as in PEGLER (1983: 47)], lamelloid hymenophore, often ciliate cap margin. No decision is made about the concept of Lentinaceae JÜLICH 1881 vs. Polyporaceae CORDA 1839.

The first author (DK) herewith proposes the following new combinations of Lentinus subgen. Lentinus species:

Polyporus phyllostipes D.KRÜGER, nom. nov.

= Lentinus crinitus (L.) FR., Syst. Orb. Veg.: 77 (1823).
Etymology: referring to gills and stipe.

Justification

The need to circumvent creation of an illegitimate later homonym to P. crinitus SPRENG. leads to the proposal of a new species epithet.


Following the arguments expressed in the CBS Aphyllumphorales database (STALPERS 2004), Polyporus crinitus SPRENG. 1820 is a legitimate name sanctioned by use in FRIES in Syst. Mycol. 1 (1821) (GREUTER et al. 2000: Art. 13.1d), as is Agaricus crinitus L. Both names are protected against any potential earlier homonyms and synonyms (GREUTER et al. 2000: Art. 15.1). For the purpose of the nomenclatural process described here, Polyporus crinitus SPRENG. is protected against any new combination. Polyporus crinitus based on a different type, such as Agaricus crinitus L. In other words, according to Article 15.2. (GREUTER et al. 2000), the epithet crinitus is unavailable for use in Polyporus because of the sanctioned name P. crinitus SPRENG. We assume that P. crinitus SPRENG. and A. crinitus L. are different from each other, hence Polyporus crinitus SPRENG. is not prorable to a potential Polyporus combination with A. crinitus L. as basionym. The epithets crinitus in P. crinitus and A. crinitus will still be available for use in other combinations.

Polyporus gerdati D.KRÜGER, nom. nov.

= (replacing the prorable but unavailable nomenclatural synonym) Agaricus tigrinus BULLIARD, Hist. Champ. France: 70 (1781).
References


BREMER, K. 1994: Branch support and tree stability. – Cladistics 10: 295–304.


FRIES, E. 1821: Systema mycologicum i. –Greifswald.


HATTORI, T. 2000: Type studies of the polypores described by E. J. H. Corner from Asia and West Pacific I. Species described in Polyporus, Buglossosporus, Meripilus, Daedalea, and Flabellrophora. – Mycoscience 41: 339–349.

HAWKSWORTH, D. L. 1984: Proposals for nomina conservanda and rejetienda for names of Hymenomycetes necessary as a result of the change in starting point for the nomenclature of Fungi. – Taxon 33: 730–736 (proposal 766).


Please refer to the original document for the complete text.


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Manuscript received: November 11th, 2003/revised version: July 12th, 2004.